



US009409950B2

(12) **United States Patent**
Miller et al.

(10) **Patent No.:** US 9,409,950 B2
(45) **Date of Patent:** Aug. 9, 2016

(54) **LINKER PEPTIDES AND POLYPEPTIDES COMPRISING SAME**

(75) Inventors: **Brian Robert Miller**, San Diego, CA (US); **Scott Glaser**, San Diego, CA (US); **Justin Caravella**, Cambridge, MA (US); **Susan Foley**, Milford, MA (US); **Xiaoping Hronowski**, Bedford, MA (US); **Tigran Aivazian**, San Diego, CA (US)

(73) Assignee: **Biogen MA Inc.**, Cambridge, MA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 288 days.

(21) Appl. No.: **13/996,918**

(22) PCT Filed: **Dec. 22, 2011**

(86) PCT No.: **PCT/US2011/066947**

§ 371 (c)(1),
(2), (4) Date: **Dec. 2, 2013**

(87) PCT Pub. No.: **WO2012/088461**

PCT Pub. Date: **Jun. 28, 2012**

(65) **Prior Publication Data**

US 2014/0079701 A1 Mar. 20, 2014

Related U.S. Application Data

(60) Provisional application No. 61/426,993, filed on Dec. 23, 2010.

(51) **Int. Cl.**

C07K 7/08 (2006.01)
C07K 16/28 (2006.01)
C07K 16/46 (2006.01)
C12N 15/62 (2006.01)
C07K 16/24 (2006.01)

(52) **U.S. Cl.**

CPC **C07K 7/08** (2013.01); **C07K 16/241** (2013.01); **C07K 16/2875** (2013.01); **C07K 16/468** (2013.01); **C12N 15/62** (2013.01); **C07K 2317/31** (2013.01); **C07K 2317/51** (2013.01); **C07K 2317/622** (2013.01); **C07K 2317/64** (2013.01); **C07K 2317/94** (2013.01); **C07K 2319/00** (2013.01)

(58) **Field of Classification Search**

CPC C07K 7/08; C07K 16/2875; C07K 16/468; C07K 16/241; C07K 2317/52; C07K 2317/569; C07K 2317/94; C07K 2317/31; C07K 2317/622; C07K 2319/00

See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

2002/0103353 A1 8/2002 Einat et al.
2008/0219947 A1 * 9/2008 Linette et al. 424/85.2

FOREIGN PATENT DOCUMENTS

WO	2005/017121	2/2005
WO	WO 2007/137279	11/2007
WO	WO 2010/010051	1/2010
WO	WO 2010/138803	12/2010

OTHER PUBLICATIONS

Robinson et al., Proc Natl Acad Sci 95: 5929-5934, 1998.*
Volkel et al., Protein Engineering 14(10): 815-823, 2001.*
Jubala et al., Vet Pathol 42: 468-476, 2005.*
Golay et al., Archives of Biochemistry and Biophysics 526: 146-153, 2012.*

Berger et al., N Engl J Med 353: 414-416, Jul. 2005.*
Getts et al., mAbs 2(6): 682-694, 2010.*
Sathish et al., Nature Reviews Drug Discovery 12: 306-324, Apr. 2013.*

International Search Report and Written Opinion in International Application No. PCT/US2011/066947, mailed Oct. 15, 2012, 26 pages.

International Preliminary Report on Patentability in International Application No. PCT/US2011/066947, dated Jun. 25, 2103, 18 pages.

Freund et al., "Characterization of the linker peptide of the single-chain Fv fragment of an antibody by NMR spectroscopy," FEBS, 1993, 320:97-100.

Hoedemaeker et al., "A single chain Fv fragment of P-glycoprotein-specific monoclonal antibody C219. Design, expression, and crystal structure at 2.4 Å resolution," J Biol Chem., 1997, 272(47):29784-29789.

Holt et al., "The Genome Sequence of the Malaria Mosquito *Anopheles gambiae*," Sci. Am. Assoc. Adv Sci., Oct. 4, 2003, 298:129-149.
Lu et al., "Bifunctional enhancement of a I-glucanase-xylanase fusion enzyme by optimization of peptide linkers," Appl. Microbiol Biotechnol., Apr. 16, 2008, 79(4):579-587.

Pihkala et al., "An antigen-mediated selection system for mammalian cells that produce glycosylated single-chain Fv," Biochem Biophys Res Comm., Nov. 26, 2004, 324(4):1165-1172.

Roch et al., "Differences in gene expression of human xylosyltransferases and determination of acceptor specificities for various proteoglycans," Biochem Biophys Res Comm., Jan. 2010, 391(1):685-691.

Schouten et al., "Improving scFv antibody expression levels in the plant cytosol," FEBS Lett., Sep. 29, 1997, 41(2):235-241.

Sheridan et al., "A new way to rapidly create functional, fluorescent fusion proteins: random insertion of GFP with an in vitro transposition reaction," BMC Neurosci, BioMed Central, London, Jun. 19, 2002, 3(1):7, 11 pages.

Volkel et al., "Optimized linker sequences for the expression of monomeric and dimeric bispecific single-chain antibodies," Protein Engineering, Oct. 2001, 14(10):815-823.

(Continued)

Primary Examiner — Phuong Huynh

(74) *Attorney, Agent, or Firm* — Fish & Richardson P.C.

(57) **ABSTRACT**

The invention is based, at least in part, on the finding that linker peptides which lack the amino acid sequence GSG reduce or eliminate the addition of posttranslational modifications to the polypeptides which comprise them. More specifically, the novel linker peptides disclosed herein reduce the ability of enzymes to link carbohydrate adducts to polypeptides comprising these linker peptides, e.g., reduce the ability of xylosyltransferase to link xylose to polypeptides. These novel linker peptides, molecules comprising same, and methods of their use are described.